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Investigation of 'Legionella pneumophila' in Drinking Water

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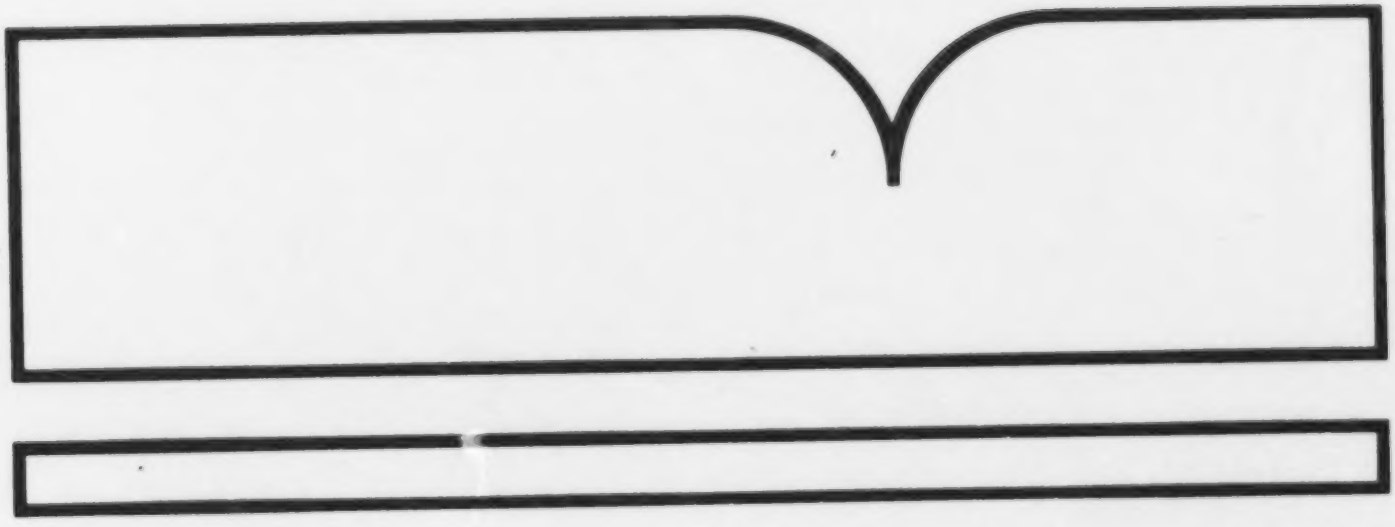

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INVESTIGATION OF *LEGIONELLA PNEUMOPHILA* IN DRINKING WATER

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16. ABSTRACT
An investigation of Legionella pneumophila in drinking water systems and home plumbing appurtenances was done in two phases. In Phase 1, 68 water samples were collected from hot and cold kitchen sink faucets in homes on 17 community water systems. No L. pneumophila organisms were isolated. In Phase 1A, the kitchen sink faucets/aerators and showerheads/supply pipes were sampled with sterile swabs and a sample of hot water was collected from hot water heaters. 184 samples (92 swabs, 92 hot water samples) were collected from homes on 4 community water systems. Two samples (1 swab, 1 hot water sample) were collected from a home with an individual, well.
Field analyses of pH, temperature, turbidity, and chlorine and laboratory analyses for standard plate count, total coliform, and iron were performed.
L. pneumophila organisms were recovered from 2 hot water heater samples; 1 from a home on a community water system and the other from the home with the well. Both positive samples were from electric, non-recirculating, hot water heaters. This finding together with the lack of recovery in the home water distribution plumbing may indicate that L. pneumophila be transported from natural aquatic sources in very low levels in drinking water systems to water heaters in homes where the organisms may increase to detectable levels. The finding that 2 of the 59 electric water heaters contained L. pneumophila may have public health significance. These sources may serve as a focus of the pathogen with resultant sporadic, endemic legionellosis.

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The complexities of environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory, Research Triangle Park, NC and Cincinnati, OH, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. Wide ranges of pollutants known or suspected to cause health problems are studied. The research focuses on air pollutants, water pollutants, toxic substances, hazardous wastes, pesticides, and nonionizing radiation. The laboratory participates in the development and revision of air and water quality criteria and health assessment documents on pollutants for which regulatory actions are being considered. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of environmental regulatory decisions involving the protection of the health and welfare of all U.S. inhabitants.

This report describes a study to determine the occurrence of Legionealla organisms in household plumbing fixtures and water systems. Information on the occurrence of this organism in such systems would be useful in assessing the potential for transmission of disease in the community through potable water.

F. Gordon Hueter, Ph.D.
Director
Health Effects Research Laboratory

ABSTRACT

An investigation of Legionella pneumophila in drinking water systems and home plumbing appurtenances was undertaken in two phases. In Phase 1, 68 water samples for L. pneumophila analysis were collected from hot and cold kitchen sink faucets in homes on 17 community water systems.

No L. pneumophila organisms were isolated from any of the samples in Phase 1. The relatively small sample size, the many variables in the sampling procedure, and potential limitations of the laboratory detection techniques employed may have contributed to the failure of significant recovery.

In Phase 1A, the kitchen sink faucets/aerators and showerheads/supply pipes were sampled with sterile swabs and a sample of hot water was collected from the drains of domestic hot water heaters in each home. A total of 184 samples (92 swabs, 92 hot water samples) were collected from homes on 4 community water systems. In addition, 2 samples (1 swab, 1 hot water sample) were collected from a home with an individual, shallow, dug well.

Field analyses of pH, temperature, turbidity, and chlorine residuals and laboratory analyses for standard plate count (SPC), total coliform, and iron content were performed using Standard Methods, 15th Edition. L. pneumophila analyses were conducted using a modification of the Gorman and Feeley Direct Plating method developed at the Centers for Disease Control.

During Phase 1A, L. pneumophila organisms were recovered from 2 hot water heater flush samples; one from a home on a community water system and the other from the home with the individual, shallow, dug well. Both of the positive samples were taken from electric, non-recirculating, hot water heaters. This finding together with the lack of recovery in the home water distribution plumbing may indicate that L. pneumophila may be transported from natural aquatic sources in very low levels in drinking water systems to hot water

heaters in homes where the organisms may increase to detectable levels.

The finding that approximately 2 of the 59 (3.4%) electric, non-recirculating, domestic hot water heaters sampled contain L. pneumophila organisms may have public health significance. These sources may serve as a foci of the pathogen with resultant sporadic, endemic legionellosis.

This report was submitted in fulfillment of Cooperative Agreement No. CR-810360 by the Vermont Department of Health under the partial sponsorship of the U. S. Environmental Protection Agency. This report covers a period from November, 1982 to March, 1984, and work was completed as of March, 1985.

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SECTION 1

INTRODUCTION/BACKGROUND

The investigation by the Centers for Disease Control (CDC) of the mysterious outbreak of pneumonia at the July, 1976 annual convention of the Pennsylvania Department of the American Legion led to the discovery of the pathogen, Legionella pneumophila (1,2). Investigations of subsequent outbreaks, together with review of stored samples from previous unexplained similar outbreaks, have added to knowledge of the pathogen and its role in human disease (3-10).

Acute human infection caused by L. pneumophila is now described as legionellosis. Legionellosis is presently recognized with two distinct clinical disease presentations: Legionnaires' disease and Pontiac fever (2,4,10,11).

Both Legionnaires' disease and Pontiac fever appear to be non-communicable; person to person transmission has not been documented (1,12). The only documented reservoir for L. pneumophila, and similar atypical L. pneumophila-like organisms, is environmental (6,8,12). Epidemiological studies have found that the method of transmission from environmental sources to susceptibles is airborne with entry into the body via the respiratory system (12).

L. pneumophila is a rod shaped bacillus measuring, 0.3 - 0.4 um in width and normally 2 - 3 um long and even 50 um in length. Electron microscopy studies have found the organisms to be atypical, gram negative bacilli, with double membranes and no cell walls (13,14,10). Some of the organisms have flagella (15).

Laboratory studies have found the organisms to be extremely fastidious bacteria with unusual growth requirements (2,16). An interesting characteristic with possible public health implications is the ability of the L. pneumophila to persist in distilled or tap water (19,20). The organisms have been found to survive up to 139 days in distilled water and for up to 369 days in tap water. In fact, one report suggests that the organism may multiply in sterile water (21).

Ecological studies suggest that the organism is part of the natural aquatic environment and is capable of surviving wide ranges of environmental conditions (16,22). In one study of 793 water samples collected from 67 different lakes and rivers in the United States, virtually all samples were found positive for L. pneumophila when the direct fluorescent antibody (DFA) technique was used for detection (23). The water samples had the following characteristics; pH 5.5-8.1, temperature 5.7-63 C, dissolved oxygen 0.3-9.6 mg/l, and Secchi disk readings 1-4 m. Chi square analysis of the data found a significantly greater isolation efficiency in the 36-60 C range. These findings suggest that L. pneumophila may favor warm aquatic environments.

Another ecological study reported the isolation of L. pneumophila from an algal-bacterial mat community growing at 45 C in association with a blue-green algae over a pH range of 6.9-7.6 (24). The researchers found that L. pneumophila was apparently using algal extracellular products as its carbon and energy sources. These observations indicate that in this particular environment of a man-made thermal effluent, the temperature, pH, and nutritional requirements were not as stringent as those observed when the organism is cultured in the laboratory on complex media. The suggestion was made that the association between L. pneumophila and certain blue-green algae explains the apparent widespread distribution of the bacterium in the natural environment.

Another possible explanation of the widespread distribution of L. pneumophila is provided by the finding that the organism can grow within freshwater and soil amoebae (25). The study found that L. pneumophila could thrive within ubiquitous soil and fresh water amoebae of the genera Acanthamoeba and Naegleria. It was suggested that amoeba-associated L. pneumophila, rather than free L. pneumophila, could be the infective form for humans.

Considering that L. pneumophila species are not new or rare organisms in the natural environment, it seems probable that a low level of previously undetected human infection with the organisms from natural sources has occurred in the past and will continue in the future. Reviews of human blood sera for DFA positivity have in some cases (Vermont studies) revealed relatively high prevalence rates of 8-26% (using a titer of 1:128 as a criterion for positivity), indicating at least a widespread human exposure to antigens consistent with those of L. pneumophila (6). Reviews have also shown seropositivity to L. pneumophila-like organisms among fatal and non-fatal clinical cases whose probable sources were aquatic (aspiration in a near-drowning, contaminated SCUBA equipment) (26). Additionally, examination of blood sera

from a large (2000) number of animals has shown evidence of widespread exposure in horses (>600 samples) to 4 serogroups of L. pneumophila (31.4% titer of > 1:64 to at least one serogroup antigen) (27).

As of September 30, 1979, 1005 confirmed cases of sporadic legionellosis in U.S. residents had been reported to CDC and 19% of these cases were fatal (28). In fact, it is estimated that there are approximately 25,000 sporadic cases of legionellosis in the U.S. per year (29). However, outbreaks of legionellosis are a relatively new occurrence. Part of the explanation for this is, of course, recognition of the disease. Yet, after discovery of the disease in 1976 with widespread media coverage and after careful historical review of information concerning past outbreaks of similar diseases it was found that the earliest recognized outbreak occurred in 1965 (30).

A review of epidemiological investigations of 17 outbreaks of legionellosis through 1979 finds that most are associated with buildings; hospitals, hotels, a golf course clubhouse, and a factory (10). Construction and excavation activities have also been implicated in some outbreaks. Cooling towers and evaporative condensers have been associated with 9 outbreaks. A history of recent travel or attendance at a convention were also factors associated with legionellosis. There were 2 outbreaks of legionellosis in Vermont in 1980 that were associated with a hospital, a medical school, and a cooling tower (31).

To date, no known outbreak of legionellosis has been directly associated with a natural environment such as a lake, pond, or stream. It appears that, in outbreak situations, human activities or man-made facilities which concentrate and/or disseminate L. pneumophila organisms in sufficient numbers and in a respirable form, are required for resultant large numbers of disease cases. A review of the environmental aspects associated with past legionellosis outbreaks may help to prevent future occurrences.

The role of buildings in legionellosis outbreaks is not clear. Certainly, buildings are necessary for bringing together large numbers of people, a critical requirement for an outbreak. There is close association between travel and/or conventions and buildings. Both of these activities usually require the use of buildings such as hotels and convention centers. In hospitals, of course, there are concentrations of people who are ill, and consequently, more at risk of contracting diseases such as legionellosis. However, not all buildings have been involved in outbreaks nor are all victims of Legionnaires' disease hospitalized. In

addition to the recognition of buildings as areas of concentrations of people, consideration must be given to activities and/or facilities in and near buildings such as construction, cooling towers, and water distribution systems, which have been associated with legionellosis outbreaks.

In 1965 an outbreak of Legionnaires' disease at a psychiatric hospital in Washington D.C. was associated with construction of a water sprinkling system on the grounds of the facility (3). There was extensive excavation undertaken on the side of the building where the epidemiological investigation found that the majority of the cases occurred. The risk factors in this outbreak included location within the hospital buildings, sleeping with open windows, and having grounds privileges.

A review of outbreaks of legionellosis through 1979 by CDC found that recent excavation or construction had been noted in 6 of the epidemics studied (32). A case-control study of sporadic cases of legionellosis found that proximity to sites of excavation for construction may be a risk factor for the disease (33). Also, this study found that construction workers had a significantly higher risk of developing Legionnaires' disease.

Construction-like activities were involved in the outbreak of Pontiac fever at James River, Virginia in 1978 (4). In that outbreak all 10 previously healthy men, 18-39 years of age, contracted Pontiac fever after cleaning a steam turbine condenser of accumulated mud, slime, and silt using compressed air blasts, resulting in the production of large numbers of aerosols. Prior to being drained for cleaning, the condenser used water from the James River for cooling and the normal discharge temperature was approximately 36 C. The environmental conditions in the steam turbine condenser are similar in many ways to cooling towers and evaporative condensers which have been involved in legionellosis outbreaks in maintenance workers and people in hotels, hospitals, and the nearby community. In 7 outbreaks, cooling towers or evaporative condensers apparently generated aerosols which contained the organism (9,10,34,35).

Cooling towers and evaporative condensers are wet type heat rejection units (WTHRU's) widely used in the modern urban and industrial environments (36). These units cool water that has been warmed by passage through industrial activities or heat exchangers used for air conditioning or refrigeration. In WTHRU's, warm water enters at the top of the unit and flows through a water distribution device which disperses the flow into small droplets which are directed down into the unit. In the unit, there may be fill material

such as wooden or plastic slats which breaks up larger droplets. The goal of the water distribution device and fill material is to create small droplets to insure maximum contact between air and water. Air may be pulled or pushed through the units by the use of fans, or natural convection may be sufficient to achieve the necessary air flow. As the water passes through the units, a portion is evaporated with resultant cooling. The cooled water is collected at the bottom of the unit and is then passed through the heat source for another cycle.

A portion of the water in WTHRU's is lost due to evaporation. In addition, a portion of the water droplets known as drift, is entrained in the airstream and lost in the exhaust plume. It is believed that aerosols in the drift loss may contain L. pneumophila organisms which may be in large numbers in the WTHRU's, due to growth and/or physical concentration.

Growth of L. pneumophila organisms may be facilitated due to environmental conditions in towers. The operating temperatures in WTHRU's are normally 30-38 C which is in the temperature range where the significantly greater isolation efficiency was reported in some of the WTHRU's associated with disease outbreaks (37).

The evaporation from WTHRU's results in the concentration of materials washed out of the air that passes through the unit. Also, materials in the make up water that is supplied to the unit to offset losses of evaporation and drift, are concentrated.

Outbreaks of legionellosis have occurred where a ventilation cross connection allowed part of an evaporative condenser exhaust to flow into the building's fresh air intake duct (34). In a hospital outbreak, it was found that the cooling tower exhaust was near the air supply intake (8). An outbreak at a hotel was associated with the exhaust plume from a positive evaporative condenser being pulled down a chimney into the meeting rooms in the hotel (38). An outbreak at a country club involved use of a practice green and two tees that were 30.5 meters from a contaminated evaporative condenser at the clubhouse (34). Two outbreaks in Vermont were epidemiologically associated with a positive cooling tower located at a medical school 150 meters from the hospital (31).

In addition to WTHRU's at or near hospitals, another environmental source of L. pneumophila organisms associated with nosocomial legionellosis is hospital water distribution systems (39-46). One report noted that the two largest sustained outbreaks of nosocomial Legionnaires' disease have been at hospitals in Los Angeles and Pittsburgh where L. pneumophila organisms have been isolated from the potable water supplies of each facility (39). Where L. pneumophila organisms have been isolated from hospital potable water distribution systems, the investigations were prompted by occurrence of nosocomial Legionnaires' disease cases.

The recovery of Legionella organisms in hospital water systems may have important public health significance because of the possible generation of contaminated aerosols from showerheads in buildings containing large numbers of immunocompromised hosts. A possible explanation of L. pneumophila organisms in hospital water systems is the normal low operating temperature (43 - 49 C) of the hot water used in these facilities (45,47,48). This temperature is used for patient safety and falls within the environmental temperature range at which optimal isolation is achieved (49,50). It is interesting to note that many other hot water systems are now being operated at similar temperatures for energy conservation reasons.

L. pneumophila organisms have also been recovered from hot water systems in non-hospital institutions and homes (50-53). While the existence of these bacteria in the plumbing systems was not necessarily associated with disease, it has been proposed that this could be a reservoir and means of transmission of legionellosis (54).

Legionella in drinking water systems has also been investigated. In a study of three municipal drinking water reservoirs, it was concluded that Legionella is either absent or present in undetectable numbers in these waters (55). In another study, 5 of 856 samples taken from chlorinated public water supplies yielded Legionella isolates (20).

In 1981 an outbreak of 34 cases of Pontiac fever and 7 cases of Legionnaires' disease was associated with exposure at one Vermont Inn (56). Environmental and epidemiological investigations were conducted to identify the sources and agents of the illness. The outbreak of Pontiac fever was caused by L. pneumophila serogroup 6. L. pneumophila serogroups 1 and 6 were identified in one environmental source at the inn, a whirlpool. After this finding, samples were collected from 5 other whirlpools at nearby inns. At 2 of the other inns, L. pneumophila was detected in whirlpools.

In the whirlpool studied, heated water (35-40 C) was circulated (3.2-4.7 l/s) through a tub in which users sat. Air was injected (3.4-5.7 m3/min) to create a swirling motion and this resulted in aerosol production (57). There are strong similarities between operation of whirlpools and WTHRUs. In a typical small cooling tower/evaporative condenser, warm water (29.40-35 C) enters at a rate of 1.9-19 l/s at the top of the unit through inlet devices with resultant water drops cascading down the unit and mixing with an air flow (57-570 m3/min) (36). This results in cooling of the water by evaporation and the production of aerosols. While air exchange rates for cooling towers/evaporative condensers are 15-1000 times greater than those of whirlpools, the human receptors of the aerosols produced in whirlpools are immersed in the tubs only centimeters above the surface of the water, while disease outbreaks have been noted at distances of 150 m from a cooling tower (31).

Another environmental finding of interest, albeit without recognized disease, is with regard to another water medium, sewage. A research project funded by the U.S. Environmental Protection Agency determined antibody levels to L. pneumophila for 433 persons living within a 1.6 km radius of an activated sludge plant (58). The antibody prevalence rate for L. pneumophila was 23.3%. In view of these findings, a search for L. pneumophila in sewage and aerosols was conducted using guinea pig passage techniques. In experiments using inoculation of undiluted sewage, L. pneumophila was detected in the animals. However, it was not possible to recover L. pneumophila when the animals were inoculated with aerosol samples. These findings suggest that viable L. pneumophila may occur in sewage. During the study, no association was noticed between these findings and overt legionellosis in the total human population of approximately 16,000 persons in the study area.

Water is an interesting and perhaps very important element common to many of the ecological findings regarding L. pneumophila organisms and environmental health investigations involved with legionellosis outbreaks. L. pneumophila organisms are found in water in the natural environment and in water in cooling towers, evaporative condensers, hospital showerheads, and whirlpools associated with legionellosis disease outbreaks. In fact, except for one report of the isolation of L. pneumophila organisms from riparian mud of a thermally polluted stream, there are no known isolations from natural environmental sources other than water (16).

Water systems may play a role in the transmission of L. pneumophila organisms with resultant legionellosis. L. pneumophila organisms common to the natural aquatic environment may be taken into water systems and delivered to devices such as cooling towers/evaporative condensers, whirlpools, showerheads, etc. In these water using devices, L. pneumophila organisms may increase in numbers due to mechanical concentration or growth resulting from favorable environmental conditions such as elevated temperature, existence of algae, amoeba, and/or other factors not yet understood. The water using devices generate aerosols which may contain L. pneumophila organisms with possible airborne transmission to hosts via the respiratory system. This scenario may explain the method of transmission of L. pneumophila organisms from the natural environment to the human host not only for outbreak situations but also for the estimated large number of sporadic cases which occur each year.

Unfortunately, there are serious questions concerning the adequacy of currently used methods of disinfecting potable water with regard to destruction of L. pneumophila. Legionella species have been isolated from chlorinated drinking water supplies and these organisms have been found to survive in tap water with and without additional sodium hypochlorite (20). A concentration of at least 0.2 mg of residual chlorine per ml was required to eliminate at least 90% of L. pneumophila in 2 hours (20). One laboratory study found chlorination at 3.3 mg/l effective against L. pneumophila (59). Another study found that Legionella organisms are much more resistant to chlorine than are coliform bacteria (60). The data in this study suggest that Legionella organisms can survive low levels of chlorine for relatively long periods of time.

A field epidemiological investigation of cooling tower water found chlorination ineffective (38). In this investigation, a cooling tower containing L. pneumophila and associated with an outbreak of Legionnaires' disease was treated with calcium hypochlorite to maintain a concentration of 3 mg/l of free available chlorine residual at a pH range of 7.5-8.5. After 7 days of treatment L. pneumophila could still be recovered from the unit.

It is obvious that more information concerning L. pneumophila in drinking water systems is required. An important concern is the method of determining the presence of L. pneumophila organisms in drinking water. The methods

commonly used for determining microbiological quality of drinking water are not suitable for determining the presence of L. pneumophila organisms. A study by the Vermont Department of Health has found no correlation between coliform and/or standard plate count results and the presence of L. pneumophila in water in cooling towers (61).

Researchers have reported the presence of L. pneumophila in drinking water using the DFA analysis technique (23). The DFA method does have limitations, however, in that there is cross reaction between L. pneumophila and at least 2 Pseudomonas strains with the DFA procedure (22). Recent attempts to overcome another problem with DFA (staining of non-viable cells) by measuring dye uptake of the electron transport system (DFA-INT) (62) do not overcome the problems of cross reactivity between L. pneumophila and Pseudomonas.

In view of the limitations of the DFA method, culture methods of analysis have been developed. The guinea pig passage (GPP) culture method was used first. However, this method is costly and, because of the laboratory containment needed, there are severe limitations on the number of analyses that can be performed in a reasonable period of time. Several culture methods using synthetic media have been developed. The Gorman-Feeley culture method using synthetic media was developed at CDC (63). A recent study comparing the GPP method with direct plating on synthetic media have shown significantly greater sensitivity of the plating method ($p<0.01$) for environmental samples (64).

During an unpublished study of WTHRU's in Vermont, one community water system, which supplied water to a cooling tower found positive on 4 occasions, was sampled and analyzed using the Gorman-Feeley method. The water system sampled was found to be positive for L. pneumophila. Of course, the detection of L. pneumophila in a drinking water system, by itself, provides little information concerning the public health significance of this finding. Epidemiological studies are required to determine the incidence of legionellosis on systems with and without L. pneumophila and other Legionella organisms.

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

In Phase 1, 68 water samples for L. pneumophila analysis were collected from hot and cold kitchen sink faucets in 2 homes on each of 17 community water systems in Vermont. No L. pneumophila organisms were isolated from any of these samples.

During Phase 1A, the kitchen sink faucets/aerators and showerheads/supply pipes were sampled with sterile swabs and a sample of hot water flushed from the drains of domestic hot water heaters in each home. A total of 184 samples (92 swabs, 92 hot water samples) were collected from homes on 4 community water systems. In addition, 2 samples (1 swab, 1 hot water sample) were collected from a home with an individual, shallow, dug well. L. pneumophila organisms were recovered from 2 samples; one from a home on a community water system and the other from the home with the individual, shallow, dug well. Both of the positive samples were taken from electric, non-recirculating, hot water heaters.

Of the 93 samples collected from domestic hot water heater tanks, 1 had fungal overgrowth which prevented completion of L. pneumophila analysis. L. pneumophila organisms were recovered from 2 of the remaining 92 samples (2.17%). The recovery of L. pneumophila from the 2 domestic hot water heaters in Phase 1A is of interest. This finding together with the lack of recovery in the home water distribution plumbing may indicate that L. pneumophila may be transported from natural aquatic sources in very low levels in drinking water systems to hot water heaters in homes where the organisms may increase to detectable levels. However, using the methods employed in this study, the occurrence of Legionella in home water systems was rare.

Continued study of this matter is recommended. Also, further information is needed regarding the sensitivity and specificity of the various laboratory methods for L. pneumophila analysis. An accurate method is needed for the isolation of L. pneumophila from environmental samples. In addition, further information regarding the epidemiology of legionellosis is required to determine the population size that must be sampled to obtain statistically significant results.

SECTION 3

METHODS AND MATERIALS

Phase 1 - It was calculated that if L. pneumophila organisms were recovered from a public water system, a minimum of 3,700 people would be required in order to conduct a subsequent statistically significant serological study to determine if such exposure could result in subclinical or clinical human legionellosis. Thus, in this phase, all water systems serving at least 3,700 people were sampled. In all, 17 systems (Barre, Bellows Falls, Bennington, Brattleboro, Burlington, Derby, Hartford, Middlebury, Montpelier, Morrisville, Newport, Northfield, Rutland, Springfield, St. Albans, St. Johnsbury and Windsor) were sampled.

Homes on each system were randomly chosen. In each home, samples for laboratory analyses for standard plate count (SPC), total coliform, L. pneumophila and iron content were collected from hot and cold water faucets on the kitchen sink. In addition, field analyses were performed to determine temperature, pH, turbidity, total chlorine residual, and free available chlorine residual.

Phase 1A - In this phase, attempts were made to isolate L. pneumophila organisms from home plumbing appurtenances and/or domestic hot water heaters in single family homes on each of 4 community water systems (Barre, Bennington, Rutland, and Windsor). A stratified number of large apartment buildings with recirculating domestic hot water systems were also sampled on each community water system (Barre-4 buildings, Bennington-5 buildings, Rutland-5 buildings, Windsor-2 buildings). Homes on each system were randomly selected. In addition one home which used a dug, shallow well as the source of water was sampled. This home was sampled because the name of the homeowner appeared on the list of customers on the Rutland City water system.

In each home, sterile swabs were used to sample kitchen sink faucets/aerators and showerheads/supply pipes. Flush samples from domestic hot water heaters were also obtained. Samples were analyzed for SPC, total coliform, L. pneumophila, and iron content. Field analyses were conducted to determine temperature, pH, turbidity, total chlorine residual, and free chlorine residual. Samples were collected over a period of 80 days and a week elapsed before returning to the same community.

FIELD METHODOLOGY

Selection Protocol

Phase 1--

1. Met with water system official and obtained billing records: recorded name, date; recorded source of names from which sites were selected;
2. Codified appropriate group from which sites were selected;
3. Picked numbers from random numbers table (30 nos./5 samples);
4. Matched numbers; recorded names, addresses, and phone numbers of private residents;
5. Contacted by telephone the homeowner(s) of the residences to be sampled and set up time for home visit; proceeded in order in which residences appeared on records until sufficient number of sites to be sampled that week had been reached; passed over any site whose owners did not answer phone or declined to permit sampling.

Phase 1A Modifications--

1. Using the water department billing list from the community to be sampled that day, sites were randomly selected.
2. The number of sites scheduled were only those that would be sampled on that day.

Sampling Protocol

General--

1. Two types of samples were taken from each of 20 households in each of 4 water systems- one type was a composite swab sample; one type was a flush sample (160 samples total).
2. Homes were stratified with hot water heated by individual heating systems, and homes with hot water heated by recirculating type systems.
3. Aerators were removed from ends of kitchen sink faucets and showerheads before sampling for swabs, and replaced after sampling was completed.

Sampling--

1. A 7-digit sample identification number (SAMID) was generated for each sample. The original 4-digit water system I.D. number (WSID) was used for first four digits. The fifth digit was the sampling round number (1 to 5). The sixth digit was the household number (1 to 4). The final digit indicated whether it was a swab sample (use a "1") or a flush sample (use a "2").
2. At the site, all sampling containers were labelled with the SAMID number.
3. A chemistry laboratory form was completed with the initial information on household.
4. Took into house:
   a. one screw-topped, sterile glass tube with 1 ml dechlorinated tap water for swab samples;
   b. one 1-gallon sterile glass bottle for flush sample;
   c. "plumbing" tools (see checklist);
   d. clipboard with questionnaire on information on hot water tanks, etc.;
   e. sterile beaker to use for collecting flush sample and transferring into 1-liter glass bottle in case bottle does not fit under drain valve;
5. Checked hot water storage tank for drain valve;

6. Swab samples
   a. Removed aerator from kitchen sink, including screen where possible;
   b. Swabbed with premoistened sterile cotton-tipped stick, around and behind aerator screen, and inside faucet pipe; placed into sterile tube;
   c. Removed aerator from showerhead of most frequently used shower;
   d. Swabbed (as in 6b) the showerhead and inside shower pipe; pooled both swabs into one sterile glass screw-topped tube;
7. Flush sample
   a. Removed metal panel in front of hot water tank that housed the temperature setting: read and recorded temperature (non-electric hot water tanks have an exposed dial with settings of warm-hot);
   b. Placed sterile 1-gallon bottle directly under hot water heater tank drain valve;
   c. Opened valve widely and let water flow immediately into bottle;
8. Took all samples and equipment outside to perform field tests.
9. Agitated flush sample by laterally shaking the gallon bottle 40 times.
10. Temperature
   a. Poured out adequate aliquot (about 25 ml) into plastic cup from 1-gallon bottle;
   b. Measured temperature with Taylor Dial Thermometer; recorded in degrees C.
11. pH
   a. Checked battery power and recalibrated meter with aliquot from 1-gallon bottle sample (see instructions enclosed with equipment);
   b. Measured and recorded pH with Digital mini-VWR Scientific Inc., pH meter, Model 55.
12. Turbidity
   a. Checked battery level and standardized nephelometer (see instructions enclosed with equipment);
   b. Used appropriate aliquots from 1-gallon bottle for measurements;
   c. Removed condensation on measuring tubes-measured and recorded in N.T.U.'s.

13. Chlorine residuals
    a. Measured and recorded both free and total chlorine residuals (using aliquots from 1-gallon bottle) with HACH Model CN-66 test kit;
    b. If chlorine residuals were present, added 2 ml aliquot of sodium thiosulfate to original sample in 1-gallon glass bottle.
14. Took sample remaining in 1-gallon bottle to Department of Health Laboratory, where various tests were performed:
    a. Completed all laboratory forms, i.e., L. pneumophila, chemical, SPC, and total coliform;
    b. Agitated 1-gallon bottle, laterally, 20 times and poured into appropriate containers for each test, using sterile technique;
    c. Aliquot of 100 ml for coliform count;
    d. Aliquot of 100 ml for standard plate count; the remaining sample was used to test other laboratory parameters (SPC, total colifrom, iron).

Transportation of Samples

1. With one exception noted below, all samples from Phase 1 were placed in an insulated chest and iced. If possible, chemical samples were stored in a separate, iced chest, since the hot water samples would warm the chest significantly.
2. The exceptions to the above were the "hot" L. pneumophila samples from phase 1. These were transported in a separate, un-iced chest, and allowed to remain at original or ambient temperature. All samples for Phase 1A were treated in this same manner.
3. The delay, or lag-time, between sampling and laboratory processing was kept to an absolute minimum. Samples were transported to the laboratory the same day of collection, before 9 p.m. (when the janitors left) or 3 p.m. on Friday (to allow processing of the SPC sample that day).
    a. Coliform and SPC samples were placed in refrigerator in the water bacteriology laboratory;
    b. Chemical and TOC samples were placed in refrigerator in the chemical laboratory;
    c. 'Cold' L. pneumophila samples were refrigerated; 'Hot' L. pneumophila samples and swab samples were kept at room temperature.

ANALYTICAL METHODOLOGY

Field analyses of pH, temperature, turbidity, and chlorine residuals were performed using Standard Methods, 15th Edition (65).

After reviewing the available methods of testing, a modification of the Gorman and Feeley Direct Plating method developed at the CDC (66) was adopted because of its sensitivity and cost-effectiveness.

Large volumes of water were needed for detection of L. pneumophila in potable water. During Phase 1, one-gallon (3.85 liters) samples were collected in sterile, non-toxic plastic containers. Phase 1A included collection of a pooled swab sample from plumbing appurtenances (kitchen sink faucet and aerator, and showerhead and shower pipe) at each home. In addition to the swab samples, a one-gallon (3.85 liter) sample of water flushed from the home hot water heater was collected. Only 1 liter of the hot water heater flush sample was analyzed for L. pneumophila; the remaining sample was used to test other laboratory parameters (SPC, total coliform, iron).

The hot water heater flush sample required special processing. Samples were placed in an ultrasonicating bath for 18 minutes to disperse and dislodge bacteria. After allowing the sediment to settle, the supernatant was removed aseptically by aspiration. Processing and isolation procedures were performed under a biological safety hood.

Water samples and environmental swabs were cultured using a modification of the Gorman Feeley direct plating method (66). The method includes concentration of water samples by filtration through a 0.2 um polycarbonate membrane filter, and direct plating on bacteriological media. Sample concentrates and sonicated environmental swab fluid were plated on buffered charcoal yeast extract (BCYE) (67,68) agar and BCYE with modified Glycine Polymyxin Vancomycin (GPV) (69) antibiotics ((Vancomycin 1 ug/ml, Polymyxin B 100 units/ml, Cyclohexamide 80 ug/ml with 0.3% Glycine. Alpha Ketoglutarate 0.1% added to media base before autoclaving (70). In order to decrease the number of non-Legionella bacteria in heavily contaminated samples an acid treatment and neutralization (66,71) process was performed on sample concentrates and environmental swab liquid. The treated samples were plated on BCYE and BCYE with antibiotics. All plates were incubated at 35 C and examined on days 3, 7, and 14. Colonies exhibiting typical Legionella morphology were

screened by streaking on BCYE and BCYE without L-cysteine-HCl. Isolates which demonstrated growth on BCYE only were considered presumptive Legionella, and were examined further by DFA test (72).

All reagents and media were prestandardized for sterility, pH and ability to support the growth of L. pneumophila. Each batch of medium was tested with a standarized suspension of guinea pig spleen inoculum containing L. pneumophila serogroup 1. A stock culture inoculum of Legionella jordanis was included as an additional control, because this is mainly an environmental organism. A known positive hot water sample containing L. pneumophila serogroup 4 and a known negative sterile tap water sample were analyzed with each set of weekly processed samples.

SECTION 4

RESULTS

Phase 1

No L. pneumophila organisms were recovered from the 68 samples collected and analyzed. Coliform and standard plate count results varied without any apparent patterns. Iron and analyses for pH, temperature, and turbidity also varied among the communities and for each sample. (See Table 1).

TABLE 1. SAMPLE ANALYSES OF PHASE 1

Water System	Date of Sample	Type of Sample (Hot or Cold)	Temp. (°C)	pH	Turb. (NTU)	Total Chlorine Residual (mg/l)	Free Chlorine Residual (mg/l)	Iron Content (mg/l)	Standard Plate Count (No./1ml)	Total Coliform (No./1ml)	Legionella pneumophila
Barre	7/12/83	cold	17.7	6.5	1.4	0.5	0.3	0.11	0	0	ND*
	7/12/83	hot	52.2	6.4	1.2	0.2	0.1	0.11	30,000	CFG*	ND
	7/21/83	cold	22.2	6.5	1.6	1.4	1.0	0.10	8	TMS*	ND
	7/21/83	hot	53.3	6.6	1.3	0.3	0.2	0.08	82	TMS	ND
Bellows Falls	7/7/83	cold	21.1	5.4	0.3	1.4	1.2	0.08	0	-	ND
	7/7/83	hot	50.6	5.7	0.4	1.0	1.0	0.06	0	0	ND
	7/25/83	cold	22.2	5.6	0.4	0.7	0.5	0	0	0	ND
	7/25/83	hot	55.6	5.9	0.4	0.2	0.1	0.16	0	0	ND
Bennington	6/23/83	cold	15.5	6.1	0.3	1.4	1.2	0	2	0	ND
	6/23/82	hot	56.1	7.8	0.2	0.1	0	0	0	0	ND
	7/27/83	cold	19.0	5.9	0.2	1.3	1.0	0	1	0	ND
	7/27/83	hot	45.0	5.9	0.6	1.1	1.0	0.10	11	0	ND
	7/27/83	cold	18.0	6.0	0.2	1.1	0.9	0.10	10	0	ND
	7/27/83	hot	83.3	6.3	0.2	0.4	0.2	0	1	0	ND
Brattleboro	6/15/83	cold	15.0	6.3	0.6	1.5	1.1	.06	0	0	ND
	6/15/84	hot	97.2	7.1	0.4	0.3	0	.07	0	0	ND
	7/20/83	cold	22.2	5.9	5.4	0.9	0.7	.14	0	0	ND
	7/20/83	hot	56.6	6.4	1.0	0.3	0.1	.14	44	0	ND
Burlington	7/1/83	cold	16.0	7.0	0.8	0.2	0.1	.46	0	0	ND
	7/1/83	hot	48.8	7.3	0.65	0.2	0.1	0.19	0	0	ND
	7/28/83	cold	21.1	7.1	1.2	1.5	1.3	0	2	0	ND
	7/28/83	hot	57.2	7.2	8.5	0.2	0	0	1	0	ND
Derby	6/21/83	cold	12.8	4.9	0.8	0	0	0.43	1	TNTC*	ND
	6/21/83	hot	47.8	6.0	2.0	0	0	0.29	0	0	ND
	7/13/83	cold	16.7	5.4	0.8	0	0	0.11	38	0	ND
	7/13/83	hot	46.7	6.4	1.2	0	0	0.17	137	0	ND
Hartford	6/27/83	cold	12.2	7.0	0.1	0	0	0	1	0	ND
	6/27/83	hot	66.7	7.5	0.2	0	0	0	2	0	ND
	7/20/83	cold	21.1	7.0	0.3	0.1	0.1	0	25	0	ND
	7/20/83	hot	80.4	7.7	0.5	0.3	0	0	0	0	ND

TABLE 1 (continued)

Water System	Date of Sample	Type of Sample (Hot or Cold)	Temp. (°C)	pH	Turb. (NTU)	Total Chlorine Residual (mg/l)	Free Chlorine REsidual (mg/l)	Iron Content (mg/l)	Standard Plate Count (No./ml)	Total Coliform (No./ml)	Legionella pneumophile
Middlebury	6/20/83	cold	12.8	6.85	1.4	0.8	0.8	0	11	0	ND
	6/20/83	hot	61.1	7.25	3.6	0.1	0	0	144	0	ND
	7/11/83	cold	16.6	7.2	0.24	0.2	0.2	0.06	0	-	ND
	7/11/83	hot	63.8	7.7	.12	0.2	0.1	0	0	0	ND
Montpelier	6/16/83	cold	15.0	7.4	0.8	0.4	0.3	0.05	0	0	ND
	6/16/83	hot	67.2	7.45	0.4	0.2	0	0.07	0	0	ND
	7/21/83	cold	16.7	7.2	1.4	0.4	0.1	0	< 1	TMS	ND
	7/21/83	hot	56.7	7.25	0.6	0.2	0.1	0.05	2	0	ND
Morrisville	6/17/83	cold	12.8	6.0	0.2	0.6	0.6	0.06	1	0	ND
	6/17/83	hot	45.0	6.0	0.5	0.7	0.6	0.05	1	0	ND
	7/12/83	cold	16	6.0	1.1	1.0	0.8	0.12	0	TNTC	ND
	7/12/83	hot	53	6.5	0.6	0.7	0.6	0	0	0	ND
Newport	7/6/83	cold	15.5	7.5	0.15	0.1	0.1	0.9	7	0	ND
	7/6/83	hot	61.0	7.5	0.60	0.1	0.1	.10	0	0	ND
	7/13/83	cold	15.6	7.5	0.1	0	0	.09	298	9	ND
	7/13/83	hot	64.4	7.7	0.1	0	0	.05	0	0	ND
Northfield	6/27/83	cold	12.2	6.1	0.25	0	0	0.18	136	TNTC	ND
	6/27/83	hot	40.6	6.5	0.25	0	0	0	20	TNTC	ND
	7/25/83	cold	11.1	6.0	0.10	0	0	0.12	0	0	ND
	7/25/83	hot	51.1	6.35	0.50	0	0	0.08	0	0	ND
Rutland	6/20/83	cold	16.1	6.15	0.4	Trace	0	0.59	2	12	ND
	6/20/83	hot	71.0	6.3	0.5	0.4	0	0.57	0	0	ND
	7/19/83	cold	20.0	6.5	0.6	2.3	2.1	0.12	1	0	ND
	7/19/83	hot	55.0	6.5	0.5	0.3	0.1	0.09	3	0	ND
Springfield	6/22/83	cold	12.2	7.3	0.1	0.3	0.2	0	0	0	ND
	6/22/83	hot	63.3	7.7	0.3	0.3	0.2	0.05	0	0	ND
	7/20/83	cold	17.8	6.9	0.35	1.7	1.5	0.10	6	0	ND
	7/20/83	hot	61.1	7.6	0.2	0.1	0	0	0	0	ND
St. Albans	6/14/83	cold	12.0	7.6	0.4	0.6	0.5	0	0	0	ND
	6/14/83	hot	60.0	7.6	0.4	0.2	0	0	11	0	ND
	7/18/83	cold	18.3	7.5	0.3	1.3	1.0	0.05	0	0	ND
	7/18/83	hot	61.1	7.6	0.4	0.2	0	0	11	0	ND
	7/26/83	cold	20.0	7.2	0.2	1.3	0.9	0	0	4	ND
	7/26/83	hot	50.0	7.4	0.3	1.1	0.9	0	0	0	ND
St. Johnsbury	7/5/83	cold	18.8	8.3	1.2	0.6	0.3	0	0	-	ND
	7/5/83	hot	58.8	7.5	3.4	0.2	0.1	0.17	0	0	ND
	7/12/83	cold	16.6	7.7	0.2	0.5	0.3	0	20	0	ND
	7/12/83	hot	52.2	7.7	0.2	0.1	0	0	0	0	ND
Windsor	7/7/83	cold	14.0	7.5	0.1	0	0	0	17,000	-	ND
	7/7/83	hot	47.0	7.2	0.9	0	0	0.09	> 20,000	0	ND
	7/25/83	cold	17.8	7.3	1.1	0	0	0	0	0	ND
	7/25/83	hot	55.6	7.3	0.5	0	0	0.18	0	0	ND

*Abbreviations: CFG (Confluent Growth); ND (None Detected); TMS (Too Much Sediment); TNTC (Too Numerous to Count)

Phase 1A

Of the 93 swab samples collected in Phase 1A, 17 had fungal overgrowth which prevented completion of L. pneumophila analyses. No L. pneumophila organisms were recovered from any of the remaining 76 swab samples collected and analyzed.

Of the 93 samples collected from domestic hot water heater tanks, 1 had fungal overgrowth which prevented completion of L. pneumophila analysis. L. pneumophila organisms were recovered from 2 of the remaining 92 samples (2.17%). One of the positive samples was from an electric, non-recirculating unit on the Barre City water system. The other positive sample was from an electric, non-recirculating hot water heater on the home with the shallow, dug well.

Counts of total coliform and standard plate varied widely among the water systems and households. No correlations can be deduced from their numbers. Drinking water systems in Barre, Bennington and Rutland were treated with chlorine routinely. No routine chlorination was provided on the Windsor water system. The individual shallow, dug well was not chlorinated. Presence of iron was also randomly found among samples. No patterns associated with iron content and water systems can be inferred from these results. Field analyses of pH, temperature and turbidity showed varying results between the water systems.

Information on the chemical, microbiological, and physical parameters of the domestic hot water heater flush samples from each water system is contained in Table 2.

TABLE 2

DOMESTIC HOT WATER HEATER TANK FLUSH SAMPLES
ANALYSES OF PHASE 1A DATA BY WATER SYSTEM

Water System	Total no. of samples	Legionella pneumophila (presence/absence)	Coliform (CFU/100ml) n min-max mean/med.	Standard Plate Count (CFU/1ml) n min-max mean/med.	Total Chlorine Residual (mg/l) n min-max mean/med.	Free Avail. Chlorine (mg/l) n min-max mean/med.	Iron (mg/l) n min-max mean/med.	pH (H+) n min-max mean/med.	Turbidity (NTU) n min-max mean/med.	Temperature (°C) n min-max mean/med.
Barre	24	1 pos. 23 ND	14 7(TMS) 2(CG) 1(TNC) 0-0 0/0	20 4(LA) 0-270,000 40,673/20	24 0.00-1.10 0.368/0.225	24 0.00-1.05 0.336/0.220	24 0.0-34.0 2.5/.28	24 6.67-8.80 7.3/7.2	24 1.3-100.0 16.6/3.5	24 22.0-69.0 38.0/37.0
Bennington	25	24 ND 1 PC	22 3(TMS) 0.00-0.00 0.00/0.00	24 1(LA) 0.000-610 49.208/0.500	25 0.000-0.900 0.095/0.001	25 0.00-0.90 0.089/0.001	25 0.000-3.400 0.386/0.130	25 4.9-8.3 6.7/6.9	25 0.28-30.00 6.25/1.40	25 22.0-74.0 41.48/43.00
Rutland	21	21 ND	11 9(TMS) 1(TNC) 0.00-0.00 0.00/0.00	20 1(TNC) 0.0-7000.00 680/2.00	21 0.000-2.100 0.484/0.100	21 0.000-2.000 .472/0.100	21 0.000-40.600 2.700/.620	21 6.12-8.68 7.13/7.04	21 .66-100.00 17.6/10.0	21 21.0-65.0 41.0/38.0
Windsor	22	22 ND	19 2(TMS) 1(TNC) 0.00-0.00 0.00/0.00	22 0.0-3000.0 491.0/6.0	22 0.000-0.000 0.000/0.000	22 0.000-0.000 0.000/0.000	22 0.000-1.110 .12/0.00	22 6.98-8.66 7.63/7.60	22 .50-17.0 3.7/1.2	22 21.0-79.0 40.0/39.0
Private	1	1 pos.	1 0	1 0	1 0	1 0	1 0	1 6.8	1 0	1 22

Abbreviations: TMS (Too Much Sediment to Test) LA (Lab Accident) CG (Confluent Growth) TNC (Too Numerous to Count) FC (Fungal Contamination) ND (Non-detected)

Information on the chemical, microbiological, and physical parameters of the positive and negative domestic hot water heater flush samples is contained in Table 3.

TABLE 3. DOMESTIC HOT WATER HEATER TANK FLUSH SAMPLES
ANALYSES OF PHASE 1A DATA BY LEGIONELLA PNEUMOPHILA RESULTS

	Legionella pneumophila (presence/absence)	Coliform Bacteria (CFU/100ml) n min-max mean/med	Standard Plate Count (CFU/ML) n min-max mean/med	Total Chlorine Residual (mg/l) n min-max mean/med	Free Available Chlorine (mg/l) n min-max mean/med	Iron (mg/l) n min-max mean/med	pH (H+) n min-max mean/med	Turbidity (NTU) n min-max mean/med	Temperature (°C) n min-max mean/med	Type of Water Heater Non-recirc. Electric	Non-recirc. Oil	Non-recirc. Gas	Non-recirc. Wood	Recirc. Electric	Recirc. Oil	Recirc. Gas	Recirc. Wood
Positive Legionella pneumophila	2	2 0.00-0.00 0/N.A.	2 4.00-3000.0 1502/N.A.	2 0.00-0.15 0.75/N.A.	2 0.00-0.10 0.050/N.A.	2 0.00-0.27 0.135/N.A.	2 6.80-7.83 7.315/N.A.	2 1.80-4.30 3.050/N.A.	2 1.80-4.30 32.5/N.A.	2	0	0	0	0	0	0	0
Negative Legionella pneumophila	90	64 0.00-0.00 0.00/0.00	87 0.00- 270000.00 9678.00/ 4.00	90 0.00-2.10 0.235/0.001	90 0.00-2.00 0.223/0.001	90 0.00-34.00 1.425/0.155	90 4.93-8.80 7.209/7.235	90 0.28-100.00 10.823/ 3.700	90 21.0-79.0 40.167/ 38.000	57	8	10	0	8	4	2	1

*Abbreviations: N.A. (Not Applicable)

Both of the positive L. pneumophila samples were recovered from electric, non-recirculating, domestic, hot water heater tanks. A wide variety of domestic hot water heaters were found as is shown in Table 4.

TABLE 4
DOMESTIC HOT WATER HEATER TYPES

	NON-RECIRCULATING	RECIRCULATING
TYPE		
Electric	59	8
Fuel oil	8	5
Gas	10	2
Wood	0	1
Total	77	16
OPERATING TEMPERATURE (oC)		
Min-Max	21 - 74	35 - 79
Mean-Med	39 - 38	46 - 43
TOTAL CHLORINE RESIDUAL (mg/l)		
Min-Max	0.000 - 2.1	0.000 - 1.5
Mean-Median	0.23 - 0.001	0.166 -0.006
FREE CHLORINE RESIDUAL (mg/l)		
Min-Max	0.000 - 2.0	0.000 - 1.4
Mean-Median	0.230 - 0.001	0.156 -0.001

SECTION 5

DISCUSSION

While several studies have shown Legionella to be a ubiquitous aquatic microorganism (16,19-26), its occurrence in drinking water systems is less clear. A study of potable water system reservoirs has shown Legionella to be either absent or present in undetectable levels in these waters (50,55,73). There are no known reports of L. pneumophila organisms being recovered directly from potable water distribution systems.

No L. pneumophila organisms were isolated from any of the 68 water samples collected from the home plumbing appurtenances during Phase 1 of this study, however, this does not necessarily mean that L. pneumophila does not exist in these environments. Another study found L. pneumophila in 3 of 856 samples taken from consumers taps on chlorinated public water systems (20). The rate of recovery of L. pneumophila in this study was 0.35% (3 of 856) which if multiplied by the total number of water samples in Phase I (68) would not result in positive findings. A more extensive sampling procedure should be undertaken before any definite conclusions can be deduced about the presence of L. pneumophila in drinking water systems.

There are several reports of recovery of L. pneumophila organisms from hot water systems in hospitals (39-46,50). The hot water systems in hospitals are normally operated at temperatures of 37.8 C - 43.3 C to prevent scalding of the patients (47-49). At these low temperatures it is necessary to rely of recirculating hot water systems, as shown in Figure 1, to maintain proper temperatures in the extremities of the systems.

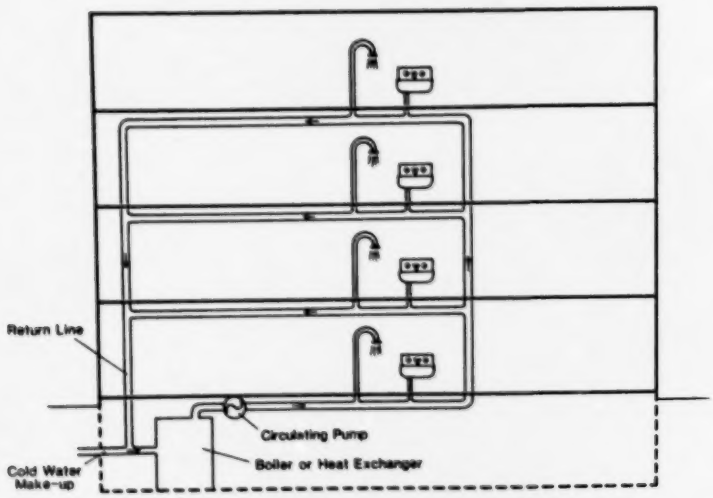

Figure 1 RECIRCULATING HOT WATER SYSTEM

The use of recirculation hot water systems may result in decreased chlorine residual levels and this in turn may allow for the survival of L. pneumophila organisms. Recirculated hot water systems are virtually nonexistent in single family homes but often used in apartment buildings to maintain proper temperatures at all points in the system. There are reports of recovery of L. pneumophila organisms from water samples collected in apartment houses (53,54).

In Phase 1A of this study there was no recovery of L. pneumophila organisms from samples collected from domestic hot water heater systems in 16 apartment buildings with recirculating hot water systems. While the range (35 C to 79 C) of the temperatures found during this study overlapped with those temperatures normally maintained in hospital hot water systems, the average temperature (46 C) was above those normally maintained in hospital systems. The systems studied in this report were small in size and 13 were able to maintain detectable chlorine residuals. Due to the small number of systems studied, it is not possible to determine any statistical significance of these findings, however more information is needed concerning the roles, if any, of chlorine and temperature in existence of L. pneumophila in recirculated hot water systems.

There is only one known report of L. pneumophila being recovered from a hot water heater in a single family home (55). This home was equipped with a side gas burner hot water heater unit, an older device which is not commonly used today. This design of this type of unit results in a space at the bottom of the tank with little circulation and a resultant accumulation of solids and lower temperatures, as is shown in Figure 2.

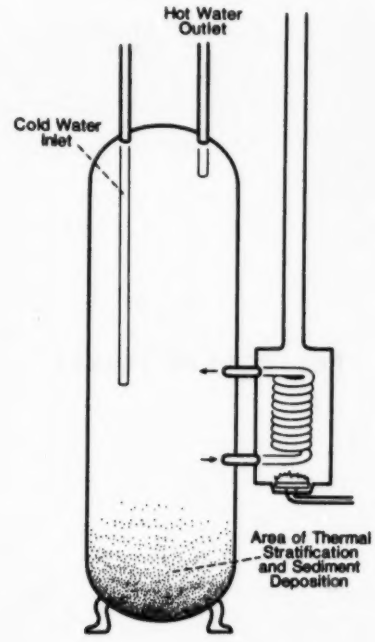

Figure 2 OLD STYLE "SIDE ARM" GAS WATER HEATER WITH TANK

In this study L. pneumophila organisms were recovered from 2 of 93 domestic hot water heaters sampled. Both of the positive units were electric hot water heaters. As shown in Figure 3, in this type of unit, normally the electric heating element is located some distance above the bottom of the tank to prevent the element from being covered with sediments and possible premature failure due to overheating. This can create an area of non-circulation at the bottom of the tank because of thermal stratification and can result in build up of sediments, lower temperatures, and reduction in chlorine residuals.

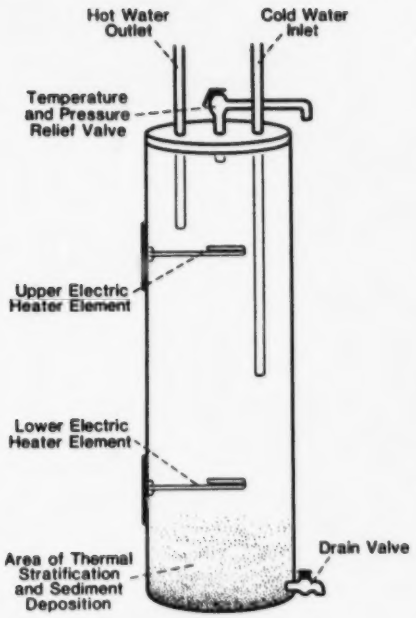
Hot Water Outlet
Cold Water Inlet
Temperature and Pressure Relief Valve
Upper Electric Heater Element
Lower Electric Heater Element
Area of Thermal Stratification and Sediment Deposition
Drain Valve

Figure 3 ELECTRIC WATER HEATER TANK

As shown in figure 4, gas hot water heaters do not have an area of non-circulation at the bottom of the tank, because of thermal mixing due to the location of the heat source.

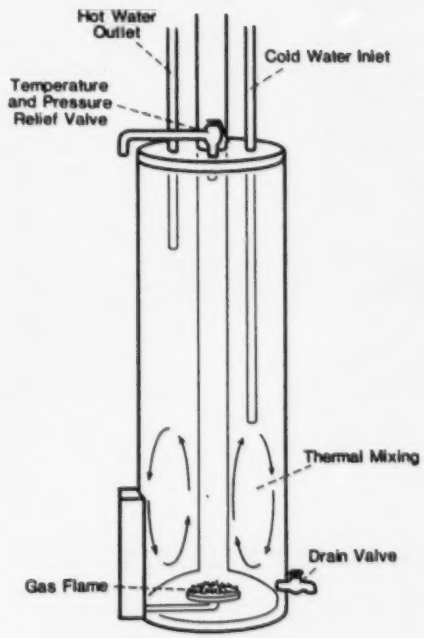

Figure 4 GAS WATER HEATER TANK

The mean temperature (32.5 C), mean turbidity (3.05 NTU), and mean SPC (1502 CFU/ml) of the positive samples were below the similar values for the negative units (mean temperature = 40 C, mean turbidity = 10.86 NTU, mean SPC = 9678 CFU/ml). One of the positive units was on an unchlorinated individual water system. The other positive unit was on a chlorinated public water system and a chlorine residual (total residual = 0.15 mg/l, free available chlorine residual = 0.1 mg/l) was detected in the sample. This recovery of L. pneumophila from a water sample with a chlorine residual is consistent with reports that the organism can survive low levels of chlorine for relatively long periods of time (58).

With potable water systems, coliform bacteria are used as surrogate organisms to determine microbiological quality (74). One purpose of this study was to determine if coliform bacteria or the SPC could be used as surrogates to determine the presence of L. pneumophila organisms. Results of

standard plate and coliform counts varied among the samples collected from home hot water heaters. Because no L. pneumophila were isolated in Phase I and there were only 2 positive samples in Phase 1A, no conclusions can be drawn regarding S.P.C. organisms and coliform as surrogates for the presence of L. pneumophila.

An in vitro study of the ecology of L. pneumophila within hot water distribution systems of hospitals found that water from a hot water storage tank readily supported the survival of L. pneumophila (75). The concentration of sediment as measured by suspended solids in this in vitro study was directly related to the survival of L. pneumophila. In our study the mean amount of sediment as measured by turbidity in the positive samples (3.05 NTU) was lower than the mean amount in the negative samples (10.823 NTU).

The in vitro ecology study also found that the presence of environmental bacteria improved the survival of L. pneumophila via nutritional symbiosis (75). The mean of the SPC (1502 CFU/ml) in our study was below the mean of the SPC values (9678 CFU/ml) of the negative values.

Isolation of L. pneumophila from hospitals (39-46,50) has been reported both with and without associated cases of legionellosis. These reports are of interest in regards to nosocomial cases of legionellosis, because of the existing health status of the persons in hospitals. There have been various reports of attempts, or suggestions on how to control L. pneumophila organisms in hospital hot water systems by increasing the temperatures maintained in the systems (46,48,50-53,76,77), reduction of sediment in the hot water systems (52,75,78), or by the use of on-site supplemental treatment to increase the levels of chlorine maintained in the systems (51,52,77,79).

These findings of this study that approximately 2 of the 59 (3.4%) electric, non-recirculating, domestic hot water heaters sampled contain L. pneumophila organisms also may have public health significance. These sources may serve as a foci of the pathogen with resultant sporadic, endemic legionellosis. As of September 30, 1979, 1005 cases of confirmed sporadic legionellosis had been reported to the Centers for Disease Control and 19% of the cases were fatal (28). A prospective study of pneumonia in Washington State found an annual incidence of 12 cases of L. pneumophila pneumonia per 100,000 population (29). Projecting this finding to the entire country, it can be estimated that there may be 25,000 sporadic cases of legionellosis per year with the potential for 4750 deaths.

In view of this more research is needed to determine the significance of the recovery of L. pneumophila in domestic hot water heaters, especially electric units. Consideration should also be given to the the importance, if any, of the areas of no circulation and the lower than average sample temperatures found for the positive units. If non-circulation is a problem a simple solution may be the extension of the cold water inlet pipe to the bottom of the tank and the use of a terminus, as shown in Figure 5, that would increase mixing to prevent thermal stratification and the build up of sediment.

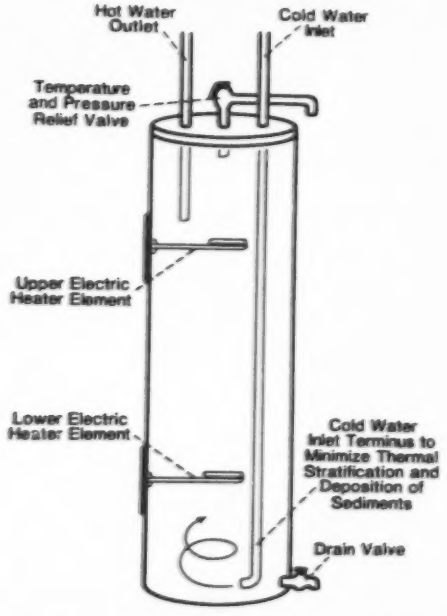
Hot Water Outlet
Cold Water Inlet
Temperature and Pressure Relief Valve
Upper Electric Heater Element
Lower Electric Heater Element
Cold Water Inlet Terminus to Minimize Thermal Stratification and Deposition of Sediments
Drain Valve

Figure 5 POSSIBLE METHOD OF MINIMIZING THERMAL STRATIFICATION AND DEPOSITION OF SEDIMENTS IN AN ELECTRIC WATER HEATER TANK

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Appendix A

TABLE A-1 PHASE 1, WATER SYSTEMS SUMMARY

System Name	Length (meters) No. of Service Connections	Source Name	Use	Type	Treatment
Barre City Water System	2800m 3000	Dix Reservoir	permanent	impoundment (surface water)	disinfection
Bellows Falls Water Dept.	1000m 1000	Minards Pond	permanent	pond (surface water)	disinfection
		Farr Brook	permanent	stream (surface water)	disinfection
		Ellis Brook	permanent	stream (surface water)	disinfection
Bennington Water Dept.	10m 3250	Bolles Brook	permanent	stream (surface water)	coagulation sedimentation mixed media filtration corrosion control disinfection
		Morgan Pond	emergency	spring (ground water)	none
		Sucker Pond	standby	lake or pond (surface water)	disinfection
		Harmon Hill Springs	emergency	spring (ground water)	disinfection
Brattleboro Water Dept.	1938m 2450	Pleasant Valley Reservoir	permanent	impoundment (surface water)	disinfection
		Retreat Meadows	permanent	gravel well (ground water)	disinfection
Burlington Water Dept.	8500m 8500	Lake Champlain	permanent	lake (surface water)	prechlorination microscreening rapid sand filtration fluoridation disinfection
Hartford Water Dept.	1183m 1562	gravel well	permanent	gravel well (ground water)	Fe & Mn removal disinfection
		Wright Reservoir	emergency	impoundment (surface water)	disinfection
International Water Co.	0m 800	Holland Pond	permanent	lake (surface water)	sedimentation disinfection
Middlebury Water Dept.	1000m 1400	Palmer Spring	permanent	gravel well (ground water)	disinfection
		Bristol Notch	permanent	rock well (ground water)	disinfection
		Palmer Spring	emergency	pond (surface water)	disinfection
Montpelier Water Dept.	194m 2900	Berlin Pond	permanent	pond (surface water)	disinfection
Morrisville Water & Light	41m 1026	Well Field	permanent	gravel well (ground water)	disinfection
Newport City Water Dept.	228m 1500	Derby Pond	standby	lake (surface water)	disinfection sedimentation

TABLE A-1(continued)

System Name	Length (meters) No. of Service Connections	Source Name	Use	Type	Treatment
Newport City Water Dept.	228m 1500	well	permanent	gravel well (ground water)	disinfection
		well	emergency	gravel well (ground water)	disinfection
Northfield Water Dept.	900m 900	well #1	permanent	gravel well (ground water)	
		well #2	permanent	gravel well (ground water)	
Rutland City Water Dept.	1900m 4975	Mendon Brook	permanent	stream (surface water)	prechlorination disinfection fluoridation
St. Albans Water Dept.	2500m 2500	Lake Champlain	permanent	lake (surface water)	prechlorination rapid sand filtration corrosion control disinfection
		Silver Lake	permanent	lake (surface water)	prechlorination coagulation sedimentation rapid sand filtration disinfection
St. Johnsbury Water Dept.	375m 2400	Styles Pond	permanent	pond (surface water)	prechlorination coagulation sedimentation mixed media filtration corrosion control disinfection
St. Johnsbury Water Dept.	375m 2400	Well	emergency	gravel well (ground water)	
Springfield Water Dept.	2200m 2200	Chapman Wellfield 1	permanent	well point (ground water)	disinfection
		Gilchrist Wellfield	permanent	gravel well (ground water)	disinfection
		Chapman Meadows II	permanent	gravel well (ground water)	
Windsor Water Dept.	50m 909	Well #1	permanent	gravel well (ground water)	none
		Well #2	permanent	gravel well (ground water)	disinfection

Appendix A

TABLE A-2 PHASE 1A, WATER SYSTEMS SUMMARY

System Name	Length (meters) No. of Service Connections	Source Name	Use	Type	Treatment
Barre City Water System	2800m 3000	Dix Reservoir	permanent	impoundment (surface water)	disinfection
Bennington Water Dept.	10m 3250	Bolles Brook	permanent	stream (surface water)	coagulation sedimentation mixed media filtration corrosion control disinfection
		Morgan Pond	emergency	spring (ground water)	none
		Sucker Pond	standby	lake or pond (surface water)	disinfection
		Harmon Hill Springs	emergency	spring (ground water)	disinfection
Rutland City Water Dept.	1900m 4975	Mendon Brook	permanent	stream (surface water)	prechlorination disinfection fluoridation
Windsor Water	50m 900	Well #1	permanent	gravel well (ground water)	none
		Well #2	permanent	gravel well (ground water)	disinfection

Appendix B

Questions for L.D.W.
Apartments and Houses Sampled

S.A.M.I.D.#

Hot water storage tank and heating water system:

1. Recirculating?

   yes
   no

2. Age of system:

3. Softeners used?

   yes What kind?
   no

4. Energy used to heat water:

   electric
   oil
   gas
   other

5. Frequency of cleaning storage tank:

6. Temperature of H_2O maintained:

In household:

1. Duration of residency:

2. Removable kitchen faucet aerator?

3. Removable shower head?

4. Number of apartments in building:

5. Number of people in residence: